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# Human iPS Cell Lines

**Cat.# SC102A-1**

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## ***User Manual***

**Store kit at -80°C on receipt**

**A limited-use label license covers this product. By use of this product, you accept the terms and conditions outlined in the Licensing and Warranty Statement contained in this user manual.**

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**List of Components**

|          |                                          |
|----------|------------------------------------------|
| SC102A-1 | Human iPS Cell Line Set<br>(feeder-free) |
|----------|------------------------------------------|

The product is shipped on dry ice and should be immediately stored in the **gas phase** of liquid nitrogen.

In general, iPS cells are challenging to culture and should only be operated by researchers experienced in the intricacies of human embryonic stem (hES) cell and human induced pluripotent (iPS) cell culture. The methods for iPS cell culture are nearly identical to hES cell culture, although more careful maintenance will be required.

## I. Human iPS Cells

### A. Description

Human induced pluripotent stem cells (iPSCs, cat# SC102A-1) were generated by transducing genetically unmodified human fibroblasts with viruses individually encoding the four human transcription factors (Oct4, Sox2, Klf4, and c-Myc) that have been shown to induce the reprogramming of somatic cells to a pluripotent state. The cells were derived using morphological selection criteria and without the use of fluorescent markers or drug selection. When cultured under standard human ES cell culture conditions, the morphology of SBI human iPSCs is identical to that of human ES cells. The cells also express the pluripotency markers Nanog, Oct4, SSEA3 and TRA-1-60, and demonstrate a strong endogenous AP activity.

Human iPS cells are adapted to feeder-free conditions and should be grown on Matrigel. Matrigel is available from BD Biosciences. PSGro iPSC Growth Media (SC500M-1) is strongly recommended as the optimal culture medium for those cells.

Human iPS cells from SBI are provided at approximately passage 10 and can be passaged 50 times before differentiation.

## B. Growth Conditions for human iPS cells

### 1. Required media and reagents

| Cat. No.       | Information                                                        |
|----------------|--------------------------------------------------------------------|
| SC500M-1       | PSGro Human ESC/iPSC Growth Medium<br>(for feeder-free conditions) |
| SC150M-1       | CRYO-GOLD Human ESC/iPSC<br>Cryopreservation Medium                |
| ZRD-Y-01/05/25 | ROCK Inhibitor (Y-27632)                                           |
| SCR005         | Accutase (Millipore)                                               |
| 354277         | Matrigel (BD Biosciences)                                          |

## 2. Coating plates with Matrigel

Matrigel (Cat. 354277, BD) should be aliquoted and stored at -80°C for long term use.

- 1) Thaw matrigel on ice until liquid. Dilute according to the manufacturer's instruction with pre-chilled Knockout DMEM/F12.
- 2) Immediately use the diluted matrigel solution to coat tissue culture-treated plates. For a 6-well plate, use 1 mL of diluted matrigel solution per well, and swirl the plate to spread the matrigel solution evenly across the surface.
- 3) Let the coated plate stand for at least 1 h at room temperature or overnight at 4°C. If plate has been stored at 4°C, allow the plate to incubate at room temperature for 1 h before removing the matrigel solution.

## 3. Thawing human iPS cells

To insure the highest level of viability, be sure to warm medium to 37°C before using it on the cells. **Due to the low survival rate of cryopreserved human iPS cells, the recovery is expected to take at least one week.**

- 1) Remove the vial from liquid nitrogen and thaw quickly in a 37°C water bath.
- 2) Remove the vial from the water bath as soon as the cells are half-thawed, and sterilize by spraying with 70% ethanol.
- 3) Transfer the cells with 10 ml of PSGro iPSC Growth Media to a 15 mL conical tube and pellet the cells by centrifugation at 200 g for 5 min.
- 4) While centrifuging, remove the matrigel solution from one well of 6-well plate. Then add 1 ml PSGro iPSC Growth Media containing 10 µM ROCK inhibitor (Y-27632).
- 5) Discard the supernatant from the human iPS cells, and resuspend the cells with 1 ml fresh PSGro iPSC Growth Media containing 10 µM ROCK inhibitor (Y-27632). Plate the cells on matrigel coated plate.
- 6) After 24 hours, remove the media and replace with fresh PSGro iPSC Growth Media (without ROCK inhibitor).
- 7) Incubate at 37°C with 5% CO<sub>2</sub> until the cells reach 80% confluency. The PSGro iPSC Growth Media must be changed every day and human iPS cells subcultured every 5-7 days.

## 4. Maintenance and Passage of human iPSCs Grown under Feeder-free condition

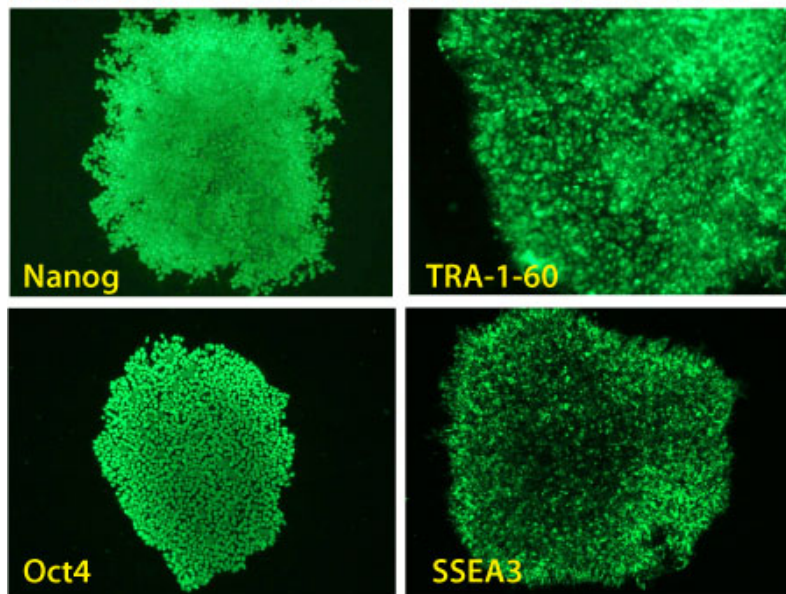
In order to maintain pluripotency, it is important to NOT keep human iPS cells in culture for long period of time without passaging.

- 1) Look under microscope to identify regions of differentiation. Mark the differentiated colonies using lens marker on the bottom of the plate.
- 2) Remove regions of differentiation by scraping with a pipette tip or by aspiration.
- 3) Aspirate medium from the hiPSC culture and rinse with DPBS (2 mL/well).
- 4) Add 0.5 mL per well of accutase (Cat. SCR005, Millipore, diluted 1:1 with DPBS before use). Let it stand at room temperature for 1-2 minutes.

- 5) Remove accutase, and gently rinse each well 1 - 2 times with 2 mL of Knockout DMEM/F-12 per well to dilute away remaining enzymes.
- 6) Add 2 mL/well PSGro Human ESC/iPSC Growth Medium and scrape colonies off with a cell scraper.
- 7) Transfer the detached cell aggregates to a 15 mL conical tube and rinse the well with an additional 2 mL PSGro Human ESC/iPSC Growth Medium to collect any remaining aggregates. Add the rinse to the 15 mL tube.
- 8) Centrifuge the 15 mL tube containing the aggregates at 200 x *g* for 5 minutes at room temperature.
- 9) Aspirate the supernatant. Resuspend pellet in PSGro Human ESC/iPSC Growth Medium containing 10  $\mu$ M ROCK inhibitor by gently pipetting and ensure that cells are maintained as aggregates.
- 10) Plate the hiPSC aggregates with PSGro Human ESC/iPSC Growth Medium onto a new plate coated with matrigel. (Remove matrigel solution before plating). *If the colonies are at an optimal density, the cells can be split every 5 - 7 days using 1:6 to 1:10 splits.*
- 11) Place the plate into the 37°C incubator and move the plate in quick side to side, forward to back motions to evenly distribute the clumps within the wells. Culture the cells at 37°C, with 5% CO<sub>2</sub> and 95% humidity.
- 12) Change medium daily.

## 5. Freezing Down iPSC Cells

- 1) Perform step 1-8 from Passaging hiPSCs Grown under Feeder-free condition
- 3) Gently aspirate the supernatant taking care to keep the cell pellet intact.
- 4) Gently resuspend the pellet in CRYO-GOLD Human ESC/iPSC Cryopreservation Medium, taking care to leave the clumps larger than would normally be done for passaging.
- 5) Transfer 1 mL of clumps in CRYO-GOLD Human ESC/iPSC Cryopreservation Medium into each labeled cryovial.
- 6) Place vials into an isopropanol freezing container and place the container at -80°C overnight.
- 7) Transfer to a liquid nitrogen tank the next day.

**C. Validation of human iPS Cells****Human iPSC Cell Line SC102A-1**

Human iPS cell colonies were stained with antibodies for stem cell markers, Nanog (upper left), Oct4 (lower left), SSEA3 (lower right) and TRA-1-60 (upper right), followed by fluorescent-labeled Alexa Fluor secondary antibodies. (Cat. No. SAB-KIT-1).

**II. References**

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### III. Technical Support

For more information about SBI products or to download manuals in PDF format, please visit our website:

<http://www.systembio.com>

For additional information or technical assistance, please call or email us at:

[tech@systembio.com](mailto:tech@systembio.com)

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